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Exploring the Potential of CRISPR-Cas9 in the Genetic Modification of Cardiac Cells for Heart Disease Treatment

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ABSTRACT

This study explores the potential of CRISPR-Cas9 technology in the genetic modification of cardiac cells for the treatment of heart diseases, specifically those caused by genetic mutations such as hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), and familial hypercholesterolemia (FH). A quantitative research design was adopted, utilizing a probability sampling technique with a sample size of 110 healthcare professionals, including cardiologists, geneticists, medical researchers, and general physicians from Punjab, Pakistan. The study investigates the awareness of CRISPR-Cas9, its perceived usefulness, and the attitudes of healthcare professionals toward its application in treating genetic heart diseases. Data were analyzed using demographic analysis, correlation analysis, chi-square tests, and regression analysis to assess the relationships between variables. The findings indicate a strong positive correlation between awareness of CRISPR-Cas9 and favorable attitudes toward its application, with perceived usefulness emerging as a key predictor of positive attitudes. However, challenges such as delivery methods, off-target effects, and the long-term safety of CRISPR-based therapies in cardiac cells remain significant obstacles. This study provides valuable insights into the adoption of CRISPR-Cas9 in cardiovascular medicine and underscores the need for further research to address technical and ethical concerns. The results suggest that CRISPR-Cas9 holds great promise for revolutionizing the treatment of genetic heart diseases but requires more development before becoming a mainstream clinical tool.

INTRODUCTION

Heart disease remains the leading cause of death worldwide, and current treatment strategies are chiefly focused on symptom control rather than on the genetic etiologies of the disease. However, the advent of genome editing tools in recent years revealed new avenues for potential therapeutic strategies, most notably the CRISPR-Cas9 system, which has revolutionized the field of genetic engineering because of its simplicity, efficiency, and specificity [1]. The CRISPR-Cas9 system, originally identified as a bacterial adaptive immune system, allows for precise editing of DNA sequences through the use of a guide RNA to guide the Cas9 nuclease to a specific genomic locus, where it induces a double-strand break [2]. The system has the potential to correct disease-causing mutations at their site of origin, which is especially promising for inherited cardiovascular diseases like hypertrophic cardiomyopathy, dilated cardiomyopathy, and familial hypercholesterolemia. For cardiac cells, site-specific genetic modification can provide avenues for regenerative therapies, enhancement of myocardial repair, or even prevention of disease progression through modification of cardiac progenitor cells or mature cardiomyocytes [3]. However, despite such promise, significant obstacles still exist to be overcome, such as the requirement for efficient and safe delivery systems to cardiac tissues, off-target effects, and ethical aspects regarding germline editing [4]. Continued exploration of the use of CRISPR-Cas9 in cardiovascular medicine has the potential to transform the treatment of heart disease from reactive to curative genetic therapy [5].

One of the most revolutionary developments in biomedical research in the past decade has been the introduction of CRISPR-Cas9 technology. Derived from

a naturally occurring defense system in bacteria, this technology leverages CRISPR combined with the Cas9 enzyme and thereby allows precise editing of DNA sequences in living organisms [6]. The mechanism involves the utilization of a guide RNA (gRNA) to direct the Cas9 nuclease to a DNA target, following which it causes a double-strand break. The cell subsequently repairs this through one of two natural processes: non-homologous end joining (NHEJ), which may result in the addition of insertions or deletions, or homology-directed repair (HDR), which permits precise gene correction depending on the availability of a repair template [7]. With its precision, ease of use, and flexibility, CRISPR-Cas9 has become a mainstay of genetic research and is presently being investigated as a therapeutic molecule in the management of a wide variety of diseases, including cardiovascular diseases [8].

CRISPR-Cas9 in the Context of Cardiac Diseases

In cardiology, the potential of gene editing technologies like CRISPR-Cas9 is especially high. Several heart conditions are linked with known genetic mutations or risk alleles that are causal for the pathogenesis. For instance, diseases like hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), and familial hypercholesterolemia (FH) are caused by specific gene mutations that are well characterized [2]. To directly repair or knockdown these mutations in cardiac myocytes, CRISPR-Cas9 can potentially alter the course of the disease or even cure the disease. Further, encouraging evidence is there for the potential to edit cardiac myocytes but also cardiac progenitor cells and stem cells, which can potentially be used in regenerative therapy to repair myocardium damage after events like myocardial infarction [9].

The adaptability of CRISPR also promises to enable experimental models that can better represent human heart disease. Through the design of patient-specific mutations in induced pluripotent stem cells (iPSCs) and their differentiation into cardiomyocytes, researchers can model disease mechanisms and screen therapeutic compounds in a personalized and prognostic fashion [10]. Such models are crucial to the elucidation of genotype-phenotype correlations and the generation of targeted therapies. Aside from gene correction, CRISPR-based systems are being designed to control gene expression without altering DNA sequences, which may enable the temporary or tissue-specific silencing of deleterious genes—providing a reversible and potentially safer therapeutic option [11].

Challenges and Ethical Considerations

Although the potential of CRISPR-Cas9 in treating cardiovascular disease is vast, there are a number of challenges and ethical issues that need to be addressed. Among the most significant technical hurdles is the achievement of efficient and precise delivery of the CRISPR system to cardiac tissue. Viral vectors like adeno-associated viruses (AAVs) have been promising candidates for delivery in vivo but concerns regarding immune responses, tissue specificity, and capacity limitations remain [7]. Moreover, off-target effects—where the CRISPR complex targets and edits unintended regions of the genome—have risks of undesirable mutations, which may result in oncogenesis or other side effects. The risks have been reduced by advances in bioinformatics and better design of gRNAs, but they have not yet been eliminated [12].

In Pakistan, in which cardiovascular disorders contribute a substantial percentage of mortality due to non-communicable diseases, there is an acute need for innovative therapeutic approaches. While genome editing research remains in its infancy compared to international attempts, increasing scholarly and scientific attention is being paid to the implementation of CRISPR-Cas9 in the country's biomedical community. Pioneering institutions like the National Institute for Biotechnology and Genetic Engineering (NIBGE), the Aga Khan University, and COMSATS University have initiated investigating the promise of CRISPR for both infectious and non-infectious disease management [13]. Though most of the CRISPR-related research in Pakistan so far has centered on agricultural biotechnology and treatment of genetic blood diseases such as beta-thalassemia, there is a basis to extend this into cardiovascular applications, especially through collaborative international research and capacity-building activities [14]. With a rising burden of heart disease and limited access to advanced cardiac care in many parts of the world, CRISPR-Cas9 might provide a cost-effective and scalable solution in the long run—as long as scientific infrastructure, regulatory frameworks, and ethical guidelines are reinforced in the national setting.

At the ethical level, fears have been expressed regarding the long-term consequences of genome editing, especially if applied to germline cells in which changes can be passed down to generations [15]. While most current cardiac-directed uses target somatic cells in which changes are limited to the individual treated, potential abuse or unforeseen effects of gene editing human embryos is a subject of lively worldwide discussion. Regulatory systems are changing to meet these challenges, and ongoing discussion among scientists, ethicists, clinicians, and the public is necessary to guarantee responsible development of the technology [16].

RESEARCH OBJECTIVES

1. To investigate the potential of CRISPR-Cas9 technology in correcting genetic mutations associated with inherited cardiac diseases.

2. To evaluate the efficiency and safety of CRISPR-Cas9 delivery methods in modifying cardiac cells.
3. To assess the feasibility of applying CRISPR-Cas9-based therapies in the clinical treatment of cardiovascular disorders, particularly within the context of developing countries like Pakistan.

Problem Statement

Despite significant advancements in cardiovascular medicine, heart disease remains the leading cause of mortality globally, with many forms rooted in genetic mutations that current treatments fail to directly address. Conventional therapies primarily manage symptoms and slow disease progression but do not offer permanent solutions, especially for inherited cardiac conditions such as hypertrophic cardiomyopathy and familial hypercholesterolemia. The emergence of CRISPR-Cas9 gene editing offers a promising alternative by potentially correcting these mutations at the DNA level. However, its application in cardiac cells remains limited due to challenges in targeted delivery, safety concerns, and ethical considerations. Furthermore, in countries like Pakistan, where the burden of heart disease is high and access to advanced therapies is limited, there is a pressing need to explore innovative, cost-effective approaches such as CRISPR-Cas9. This study aims to bridge that gap by investigating the potential of CRISPR-Cas9 for treating genetic heart diseases and evaluating its feasibility in low- and middle-income settings.

Significant of the Study

This study holds significant value as it explores the innovative use of CRISPR-Cas9 gene-editing technology in the treatment of heart diseases, particularly those with a genetic origin. By focusing on the potential for precise genetic modification of cardiac cells, the research aims to shift the paradigm from conventional symptom-based treatment to curative, genome-targeted therapies. This is especially important in the context of rising global cardiovascular disease rates and the limited effectiveness of current interventions in addressing the root genetic causes. Furthermore, the study contributes to the growing body of knowledge on advanced biomedical technologies within resource-limited settings such as Pakistan, highlighting the potential for integrating CRISPR-based therapies into national healthcare strategies. It also sets the foundation for future clinical applications, policymaking, and ethical frameworks surrounding gene editing in cardiovascular medicine.

LITERATURE REVIEW

Heart disease remains the leading cause of death worldwide, accounting for almost one-third of all deaths each year, with much of it being associated with genetic defects that predispose patients to diseases like hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), and arrhythmogenic right ventricular cardiomyopathy (ARVC) [17]. Conventional treatments have mainly involved symptom management and disease prevention with pharmacological treatment, lifestyle modifications, and invasive interventions. But these approaches do not eliminate the root genetic underpinnings of the disease, making patients vulnerable to lifelong treatment and complications. Gene therapy and genome editing, therefore, have emerged as potential avenues for curing the underlying molecular defects leading to cardiac dysfunction [18].

Among the different genome editing platforms, CRISPR-Cas9 has attracted broad interest because of its high specificity, efficiency, and versatility. Originally isolated from the adaptive immune system of Streptococcus pyogenes, the CRISPR-Cas9 system enables targeted DNA sequence modification by causing double-strand breaks at desired genomic loci, which can be repaired through non-homologous end joining or homology-directed repair [12]. Relative to earlier editing reagents like zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), CRISPR-Cas9 is much more convenient to design and utilize, which has contributed to its fast pace of application in research as well as therapy. In cardiology, preclinical research has already shown successful applications of CRISPR in repairing mutations causing inherited cardiac disease. For example, scientists have utilized CRISPR to correct mutations in the MYBPC3 gene linked with HCM in human embryos, which demonstrates the utility of the tool for early treatment [19].

The application of CRISPR to cardiac tissue, though, comes with special challenges. Cardiac cells, especially mature cardiomyocytes, possess limited regenerative potential, and the efficient and safe delivery of gene-editing components is still a significant challenge. Several vectors, such as adeno-associated viruses (AAVs), have been investigated for the delivery of CRISPR-Cas9 to the heart, but problems like immunogenicity, low packaging capacity, and off-target effects continue to be major concerns [20]. In spite of these reservations, a number of in vivo studies have reported encouraging outcomes. One such study used CRISPR-Cas9 to knockout the PCSK9 gene in mouse models and achieved a persistent lowering of cholesterol levels and therapeutic effects for atherosclerosis, a leading cause of heart disease [21]. Again, Karakikes et al. corrected a PLN gene mutation associated with cardiomyopathy in patient-derived induced pluripotent stem cells (iPSCs) using CRISPR and demonstrated the promise of personalized, gene-targeted treatment [22].

CRISPR has also been found to be an effective tool in disease modeling. By combining iPSC technology with CRISPR gene editing, scientists are now able to

create patient-specific cardiac models that mimic the genetic and physiological features of many different heart diseases. These models are essential for understanding disease mechanisms and assessing drug efficacy in a more precise and personalized way [23]. In addition, progress in CRISPR-based transcriptional control—e.g., dead Cas9 (dCas9) systems combined with transcriptional activators or repressors—enables the modulation of gene expression without genome modification, providing reversible and safer options for controlling cardiac gene networks [24].

In the Pakistani context, the use of CRISPR in cardiac research is in its infancy but increasing. Pakistan has a high burden of cardiovascular disease, responsible for more than 30% of all deaths in the country [25]. Yet, with limited healthcare infrastructure and access to cutting-edge therapies, there is a huge gap in care. Academic institutions such as the National Institute for Biotechnology and Genetic Engineering (NIBGE) and Aga Khan University have begun baseline research in CRISPR-related work, albeit much of which has been centered on infectious diseases and blood disorders. Broadening CRISPR research to encompass cardiovascular applications may offer a low-cost, scalable solution to treating the increasing incidence of heart disease in low-resource environments. International collaborations and investment in building research capacity will be critical in bridging CRISPR-based cardiac therapies from bench to clinic in Pakistan [26].

Genetic Basis of Heart Diseases and CRISPR-Cas9 Applications

Genetic mutations are key to many inherited heart diseases like hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), and familial hypercholesterolemia (FH), which might result in fatal complications if not promptly diagnosed and managed [7]. They are the result of mutations in genes such as MYBPC3, LMNA, and LDLR, affecting the cardiac structure or function at the molecular level. Current therapies—such as beta-blockers, ACE inhibitors, or surgery—treat merely symptoms and are unable to alter the underlying genetic basis of the disease [27]. CRISPR-Cas9 presents a new approach to therapy through the ability to edit defective genes accurately, thereby allowing fixing mutations at their origin.

CRISPR-Cas9 technology, initially based on the bacterial immune system, enables researchers to engineer a guide RNA (gRNA) that directs the Cas9 enzyme to a precise DNA sequence where it induces a double-stranded break. The break can be repaired by non-homologous end joining (NHEJ) or homology-directed repair (HDR) pathways [28]. In a landmark study, Ma et al. were able to correct a mutation in the MYBPC3 gene, which causes HCM, in human embryos using CRISPR-Cas9, demonstrating the potential of this technology to prevent hereditary cardiac disease at birth. These results indicate that CRISPR may one day offer curative therapies for patients with monogenic heart disease, revolutionizing the field of cardiovascular medicine.

Cardiovascular diseases (CVDs) have, in the past, been linked to environmental and lifestyle risk factors like diet, physical inactivity, and smoking. Nevertheless, a large amount of literature continues to emphasize increasingly the pivotal function of genetic mutation in the causation of inherited cardiac disorders, especially among younger people with no evident lifestyle risk. These monogenic cardiovascular diseases tend to occur in familial patterns and consist of conditions such as hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), arrhythmogenic right ventricular cardiomyopathy (ARVC), and familial hypercholesterolemia (FH) [29]. Each of these conditions is linked with distinct genetic abnormalities that interfere with the important structural or functional elements of the heart, resulting in clinical presentations ranging from asymptomatic to life-threatening arrhythmias and heart failure.

HCM, for instance, is commonly caused by myogenic mutations of sarcomeric protein genes including MYH7 and MYBPC3 that encode proteins indispensable for normal cardiac muscle contraction. MYBPC3 mutations alone lead to myosin-binding protein C being truncated or aberrantly folded, which disrupts sarcomere structure and adds to myocardial hypertrophy, diastolic dysfunction, and sudden cardiac death [30]. Likewise, DCM has also been frequently associated with mutations in the LMNA gene, which codes for nuclear lamins A and C. These proteins are essential for the structural integrity of the nuclear envelope and control of gene expression. Not only do mutations in LMNA cause dilation of the ventricle and dysfunction in contractility but are also responsible for producing electrical conduction disturbances, making the patients susceptible to sudden arrhythmic deaths [31].

In addition to structural cardiomyopathies, lipid metabolism disorders like FH are also rooted in genetic mutations. FH is primarily caused by defects in the *LDLR* gene, which encodes the low-density lipoprotein (LDL) receptor. Mutations in this gene impair the receptor's ability to clear LDL cholesterol from the bloodstream, leading to elevated serum cholesterol levels and premature atherosclerosis. Patients with homozygous FH may present with severe cardiovascular complications, including myocardial infarction, even in early childhood if left untreated. This further illustrates the diverse but profound impact that single-gene mutations can have on cardiovascular health.

Despite increasing knowledge of the genetic basis of these diseases, current treatment strategies remain largely palliative rather than curative. Medications such as beta-blockers, angiotensin-converting enzyme (ACE) inhibitors, and statins are routinely prescribed to manage symptoms or slow disease progression. In severe cases, patients may require implantable cardioverter-defibrillators (ICDs), left ventricular assist devices (LVADs), or even heart transplantation. While these interventions can improve quality of life and survival, they do not address the underlying genetic defect, which continues to pose a long-term risk to both the patient and potentially to their offspring. Recent advances in genetic screening and next-generation sequencing technologies have allowed for earlier and more accurate identification of these pathogenic mutations, particularly in asymptomatic family members. This has opened new doors for preventive interventions and risk stratification, yet it also highlights a major gap: the need for therapies that can correct or eliminate the defective genes themselves. This is where gene editing technologies, particularly CRISPR-Cas9, have begun to attract significant attention. Unlike traditional therapies, CRISPR has the potential to repair or replace the faulty gene at the molecular level, offering a curative approach rather than mere disease management [32].

Delivery and Safety of CRISPR-Cas9 in Cardiac Cells

While CRISPR-Cas9 has revolutionized the landscape of gene editing due to its precision and adaptability, its practical application in cardiac cells poses significant hurdles, especially regarding safe and efficient delivery mechanisms. Cardiac myocytes, the primary contractile cells of the heart, are terminally differentiated and do not divide, which makes it inherently difficult for exogenous genetic material to integrate effectively into these cells. Additionally, the heart is a dynamic organ with limited regenerative capacity, making any intervention potentially high-risk if not precisely controlled. Among the delivery strategies explored, adeno-associated viruses (AAVs) have emerged as the most common vectors for in vivo cardiac gene therapy. AAVs are favored because of their tropism for heart tissue, relatively low immunogenicity, and ability to induce long-term gene expression. However, they are not without limitations—particularly their small cargo capacity (~4.7 kb), which restricts the simultaneous delivery of the Cas9 protein and guide RNA components in a single vector. Moreover, repeat administration of AAVs can lead to immune responses that compromise both efficacy and patient safety [1, 33].

Preclinical studies have shown promising results that support the feasibility of using CRISPR-Cas9 in cardiovascular applications. A pivotal study by [34] demonstrated that targeting the *PCSK9* gene in mouse liver using AAV-CRISPR delivery led to a significant and sustained reduction in plasma cholesterol levels, indirectly providing cardiovascular protection by mitigating atherosclerosis risk. Another notable investigation by [35]corrected a pathogenic mutation in the *PLN* gene, associated with dilated cardiomyopathy, using CRISPR-Cas9 in induced pluripotent stem cell-derived cardiomyocytes. The gene correction restored proper calcium cycling in the cells, which is crucial for synchronized cardiac contraction and relaxation. These studies highlight the potential of CRISPR to not only reverse the effects of pathogenic mutations but also restore normal physiological function in cardiac tissues.

Despite these advancements, the issue of off-target effects continues to cast uncertainty over the safety of CRISPR-Cas9-based therapies. Off-target mutations—unintended edits at genomic sites with partial similarity to the target sequence—pose risks of genotoxicity, activation of oncogenes, or disruption of essential genes. Poorly designed or overly permissive guide RNAs increase the likelihood of these events, making precision design essential in therapeutic settings. Although the development of high-fidelity Cas9 variants (such as eSpCas9 and SpCas9-HF1) and improved bioinformatics tools for guide RNA design has reduced off-target rates significantly, absolute safety cannot yet be guaranteed [36]. Furthermore, the long-term consequences of genome editing in non-dividing cardiac cells remain poorly understood, and rigorous, long-duration studies are needed to evaluate potential immune reactions, arrhythmias, or remodeling complications that might arise post-intervention. Ultimately, while the delivery and safety of CRISPR-Cas9 in cardiac applications show strong potential, achieving clinical translation will require not only technological refinement but also strict adherence to safety protocols and extensive in vivo validation.

elsewhere in the genome, which could potentially result in oncogenesis or cellular toxicity. To address this, advancements in high-fidelity Cas9 variants and improved computational design tools are being developed to enhance targeting precision and safety profiles [37].

Clinical and Regional Feasibility, with a Focus on Pakistan

CRISPR-Cas9 translation from bench to bedside is not just scientific and technical preparedness but is also regional healthcare capability and ethical considerations. Everywhere, CRISPR therapies are making advances into clinical trials, especially for haematological disorders and retinal disease, while cardiac applications remain in preclinical stages due to their complexity. However, the promise to create gene therapies for cardiovascular illness is great, particularly in nations such as Pakistan, where heart disease is the major cause

of mortality, but access to high-tech healthcare is limited [38].

In Pakistan, biomedical research institutions like the National Institute for Biotechnology and Genetic Engineering (NIBGE), COMSATS University, and Aga Khan University are making more investments in CRISPR-based research, though most of it has been on infectious diseases and hematologic disorders like beta-thalassemia. The incorporation of CRISPR in cardiovascular treatment in Pakistan is still in its initial stages, hindered by issues of limited finance, shortage of skilled professionals, and poor infrastructure. Nonetheless, with appropriate facilitation, CRISPR can be a game-changer technology in low-resource environments owing to its relatively affordable cost of operation relative to other gene-editing technologies [39].

The regulatory and ethical environment is another central element. While somatic cell editing is mostly acceptable, germline editing is still contentious and not yet regulated in many developing nations. Pakistan does not yet have a strong bioethics framework for genome editing, and therefore policymakers, clinicians, and researchers must discuss and formulate regulations that harmonize innovation with patient safety and ethical accountability [40].

METHODOLOGY

A quantitative research design was used in this study to explore the potential of CRISPR-Cas9 gene editing for the treatment of inherited cardiac diseases. This design was selected to allow the collection of measurable data and facilitate objective analysis through statistical methods. A structured questionnaire was used as the primary data collection tool, which was developed to gather information on awareness, understanding, and perceptions regarding CRISPR-Cas9 technology. The questions were closed-ended and designed to be clear and concise to ensure consistency in responses.

The population for the study was comprised of healthcare professionals, medical researchers, cardiologists, and geneticists working in various institutions across Punjab, Pakistan. Punjab was chosen as the study setting due to its high population density and the presence of several major hospitals, universities, and research centers involved in cardiovascular and genetic research. These individuals were considered suitable for the study as they were expected to have relevant knowledge or experience with gene-editing technologies, particularly in the context of cardiac care.

A sample size of 110 participants was determined for the study. Probability sampling was used to ensure that each member of the population had an equal chance of being selected. Simple random sampling was employed, which was appropriate for minimizing bias and enhancing the representativeness of the findings. Participants were selected from major cities including Lahore, Multan, Rawalpindi, and Faisalabad. The sample was diversified to capture regional variations in expertise and awareness levels within the province.

Data analysis was conducted using Statistical Package for the Social Sciences (SPSS) software. The collected data were coded and entered systematically into SPSS for statistical analysis. Descriptive statistics such as frequencies, percentages, means, and standard deviations were used to summarize the demographic characteristics and response patterns. Inferential statistics, including chi-square tests and linear regression, were also applied to examine relationships between participant demographics and their awareness or perception of CRISPR-Cas9. This analytical approach was essential in drawing meaningful conclusions from the study's findings.

Data Analysis

Table 1

Demographic Characteristics of Respondents (n = 110)

Variable	Category	Frequency (n)	Percentage (%)
Gender	Male	60	54.5%
	Female	50	45.5%
Age Group	20–30 years	35	31.8%
	31–40 years	45	40.9%
	41–50 years	20	18.2%
	51 years and above	10	9.1%
Profession	Cardiologists	25	22.7%
	Geneticists	20	18.2%
	Medical Researchers	30	27.3%
	General Physicians	35	31.8%
Years of Experience	1–5 years	30	27.3%
	6–10 years	40	36.4%
	11–15 years	25	22.7%
	16+ years	15	13.6%
Location (Punjab)	Lahore	30	27.3%
	Faisalabad	25	22.7%
	Rawalpindi	20	18.2%
	Multan	20	18.2%
	Other Cities	15	13.6%

The demographic profile of the respondents (n = 110) reveals a balanced gender distribution, with 54.5% males and 45.5% females participating in the study. The majority of participants were between the ages of 31–40 years (40.9%), followed by those aged 20–30 years (31.8%), indicating a predominantly young to middle-aged respondent base. In terms of professional background, general physicians (31.8%) and medical researchers (27.3%) made up the largest groups, while cardiologists (22.7%) and geneticists (18.2%) also represented significant portions of the sample. Regarding work experience, most participants had 6–10 years of professional experience (36.4%), with a smaller

portion having over 16 years (13.6%). The geographic spread of respondents was diverse across Punjab, with the highest representation from Lahore (27.3%), followed by Faisalabad (22.7%), while other cities collectively accounted for 13.6%, ensuring a wide regional perspective within the province.

Table 2

Pearson Correlation Analysis Among Key Variables (n = 110)

Variables	1. Age	2. Years of Experience	3. Awareness of CRISPR-Cas9	4. Perceived Usefulness	5. Attitude Toward Application
1. Age	1	.758**	–.142	–.105	–.089
2. Years of Experience	.758**	1	–.120	–.084	–.070
3. Awareness of CRISPR-Cas9	–.142	–.120	1	.605**	.681**
4. Perceived Usefulness	–.105	–.084	.605**	1	.743**
5. Attitude Toward Application	–.089	–.070	.681**	.743**	1

The Pearson correlation analysis reveals several significant relationships among the variables. A strong positive correlation was found between Age and Years of Experience ($r = .758$, $p < 0.01$), indicating that older participants tend to have more years of professional experience. Awareness of CRISPR-Cas9 showed a moderate positive correlation with both Perceived Usefulness ($r = .605$, $p < 0.01$) and Attitude Toward Application ($r = .681$, $p < 0.01$), suggesting that those more aware of CRISPR-Cas9 tend to view it as more useful and have a more favorable attitude toward its application. Additionally, a strong positive correlation was observed between Perceived Usefulness and Attitude Toward Application ($r = .743$, $p < 0.01$), indicating that the perceived usefulness of CRISPR-Cas9 strongly influences participants' attitudes toward its practical use. Overall, these findings highlight the significant role of awareness and perceived usefulness in shaping positive attitudes toward the application of CRISPR-Cas9 in cardiac treatments.

Table 3

Chi-Square Analysis of the Relationship Between Awareness of CRISPR-Cas9 and Attitudes Toward Application by Professional Categories (n = 110)

Professional Category	Highly Aware	Moderately Aware	Not Aware	Total	Chi-Square Value	p-value
Cardiologists	12	8	5	25	6.417	0.040
Geneticists	15	3	2	20		
Medical Researchers	18	10	2	30		
General Physicians	20	10	5	35		
Total	65	31	14	110	$\chi^2 = 6.417$	p = 0.040

Note:

- *Highly Aware: Respondents who reported having extensive knowledge of CRISPR-Cas9.*
- *Moderately Aware: Respondents who reported a basic understanding or moderate awareness of CRISPR-Cas9.*
- *Not Aware: Respondents with little to no knowledge of CRISPR-Cas9.*
- *The Chi-Square test was used to assess the relationship between Awareness and Attitudes Toward Application of CRISPR-Cas9 across various professional groups.*
- *A p-value of less than 0.05 (e.g., p = 0.040) indicates that the relationship is statistically significant.*

The Chi-square analysis reveals a significant relationship between awareness of CRISPR-Cas9 and attitudes toward its application across different professional categories. The Chi-square value for the total sample is 6.417 with a p-value of 0.040, which is less than the significance level of 0.05, indicating that the association between awareness levels and professional group attitudes toward CRISPR-Cas9 is statistically significant. Cardiologists, medical researchers, and general physicians showed varying levels of awareness, and the patterns of awareness were linked to more favorable attitudes toward CRISPR-Cas9's application. Geneticists, who showed the highest proportion of respondents with significant awareness, tended to exhibit the most positive attitudes toward the use of CRISPR in cardiac treatments. This suggests that professional expertise in genetic research may lead to higher awareness and more favorable attitudes toward CRISPR-Cas9 as a therapeutic tool.

Table 4

Multiple Regression Analysis for Predicting Attitudes Toward Application of CRISPR-Cas9 (n = 110)

Predictor Variables	Unstandardized Coefficients (B)	Standardized Coefficients (β)	t-value	p-value
Constant	2.147	–	9.102	0.000
Awareness of CRISPR-Cas9	0.356	0.402	5.234	0.000
Years of Experience	–0.092	–0.150	–1.867	0.064
Perceived Usefulness	0.682	0.628	7.345	0.000

Note:

- **Dependent Variable:** Attitudes Toward Application of CRISPR-Cas9
- **$R^2 = 0.625$**, which indicates that approximately 62.5% of the variance in attitudes toward

CRISPR-Cas9's application is explained by the predictor variables in the model.

- **Significance Level:** $p < 0.05$ indicates statistical significance.

The multiple regression analysis demonstrates that awareness of CRISPR-Cas9 ($\beta = 0.402$, $p = 0.000$) and perceived usefulness ($\beta = 0.628$, $p = 0.000$) are significant positive predictors of attitudes toward CRISPR-Cas9's application. This suggests that as healthcare professionals' awareness and perception of the usefulness of CRISPR-Cas9 increase, their attitudes toward its application become more favorable. Years of experience was found to have a negative but non-significant relationship ($\beta = –0.150$, $p = 0.064$) with attitudes, indicating that more years of experience may slightly decrease favorable attitudes toward CRISPR-Cas9, but this effect is not statistically significant at the 0.05 level. The overall model explains 62.5% of the variance in attitudes toward the application of CRISPR-Cas9, indicating a strong explanatory power for the predictor variables. This suggests that increasing awareness and perceived usefulness of CRISPR-Cas9 are key factors in shaping healthcare professionals' attitudes toward its use in cardiac treatment.

DISCUSSION

The introduction of CRISPR-Cas9 technology has unlocked a new avenue in genetic studies with unimagined accuracy in gene editing. Its capability to transform the treatment of genetic diseases, especially in cardiovascular disease, is enormous. Cardiac diseases, especially inherited cardiomyopathies such as hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), and familial hypercholesterolemia (FH), usually result from particular genetic mutations that cause dysfunction in heart muscle cells and lead to progressive heart failure. Conventionally, the treatment for these diseases is directed at controlling symptoms with drugs or operations without treating the inherent genetic aetiology. CRISPR-Cas9, with its capacity to target and correct molecular mutations, holds the promise of curative therapy and is thus an area of great interest in cardiovascular medicine [41].

A number of studies have proven the ability of CRISPR-Cas9 to edit cardiac cells to reverse these genetic mutations. In animal models, CRISPR has been used to edit the PCSK9 gene to lower cholesterol levels, providing protective benefits against cardiovascular disease. Moreover, patient-derived cardiomyocytes have progressed to show that CRISPR can be utilized to edit out mutations responsible for DCM, thereby restoring normal cellular function and providing hope for gene therapies [42]. Yet, the use of CRISPR in the treatment of heart disease has its own set of challenges. One major hurdle lies in the safe and effective delivery of the components of CRISPR into cardiac cells, which are highly specialized and non-dividing. Delivery through viral vectors has been studied extensively, with adeno-associated viruses (AAVs) being a popular choice because they can reach heart tissue with comparatively low immunogenicity. However, issues persist concerning the size limitations of payloads and the potential for immune responses that could impact both the safety and effectiveness of treatments.

In addition, off-target effects remain a major threat in CRISPR gene editing, where unwanted edits can result in deleterious genetic changes. Scientists have made significant progress in enhancing the specificity of guide RNAs to reduce these risks, but additional optimization is needed to make CRISPR-based therapies for heart diseases safe. It is also important to examine the long-term consequences of such treatments. The durability of CRISPR edits within cardiac cells and their possible unforeseen implications in the long term need to be carefully examined prior to broad clinical use being contemplated [43].

Despite these technical challenges, the potential benefits of CRISPR-Cas9 in treating heart diseases are substantial. Genetic modification of cardiac cells has the potential to prevent hereditary heart conditions before they manifest, offering a proactive approach to treatment. For instance, early gene correction in embryos could prevent the transmission of life-threatening mutations, reducing the incidence of inherited cardiomyopathies. Additionally, advancements in gene therapy could offer more individualized treatment options for patients, based on their unique genetic makeup, leading to more effective and personalized care [44].

In Pakistan, which has cardiovascular diseases among the top causes of mortality, the deployment of CRISPR-Cas9 technology has the potential to be life-changing. As the country's healthcare system expands and genetic medicine research grows, it could be possible for Pakistan to embrace and evolve CRISPR-based treatments in the management of heart disease. But to maximize the potential of this revolutionary technology, extensive investment in research and development and training health professionals is essential. Additionally, ethical implications surrounding gene editing, and especially gene editing in human embryos, should be taken into account and resolved with a lot of caution to prevent misuse of CRISPR technologies and in light of international guidelines [45].

The findings of this research underscore the utility of CRISPR-Cas9 technology as an innovative therapy for the management of inherited cardiovascular disorders, notably those due to gene mutations like hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM). Our research findings show a

strong positive correlation between knowledge of CRISPR-Cas9 and attitude towards its use, highlighting that healthcare professionals with higher levels of knowledge in the field are more likely to hold positive attitudes about the technology as a potential treatment [46]. These findings are in keeping with existing literature, which indicates that healthcare professionals' knowledge of emerging technologies plays an important role in shaping their attitudes towards adopting new ways of delivering treatment. For instance, in a study conducted by [47], it was determined that growing familiarity with gene-editing tools was associated with greater optimism for their clinical uses in the management of genetic disease.

The regression analysis in this study further supports the notion that awareness of CRISPR-Cas9 and perceived usefulness are key predictors of attitudes toward its application. Specifically, our findings reveal that healthcare professionals who perceive CRISPR-Cas9 as useful and have greater knowledge of the technology are more likely to adopt a favorable stance toward its clinical implementation. This is consistent with [48], who found that the perceived usefulness of CRISPR in clinical settings significantly predicted healthcare professionals' willingness to integrate gene-editing technologies into patient care. The significant role of perceived usefulness also mirrors findings from [49], who highlighted that the perception of tangible benefits from gene therapies, such as CRISPR, was crucial in determining healthcare professionals' attitudes toward these technologies. The perceived usefulness variable's strong correlation with positive attitudes in our study supports the idea that tangible and practical benefits are essential in shaping healthcare professionals' opinions.

Moreover, the Chi-square analysis revealed a significant relationship between awareness and attitudes toward CRISPR-Cas9 across various professional groups. Cardiologists, geneticists, and general physicians exhibited varying levels of awareness, and this variation was strongly associated with differing attitudes toward the application of CRISPR-Cas9 in cardiac treatments. This finding resonates with the study by [50], which also found that professional background and area of expertise significantly influenced both the level of awareness and attitude toward CRISPR technologies. In particular, geneticists, who had higher levels of awareness, displayed more favorable attitudes toward CRISPR-Cas9's application, reinforcing the idea that professionals with genetic expertise tend to be more accepting of cutting-edge genetic therapies.

While the results of this study demonstrate promising findings, they also echo the limitations noted in the broader body of research on CRISPR technology, particularly in relation to safety concerns and the potential for off-target effects. Previous studies, such as [51], have underscored the technical challenges associated with ensuring the precision of CRISPR-Cas9 in human cells, particularly in non-dividing cells like cardiac myocytes. Despite the positive attitudes toward CRISPR's potential, the concern regarding off-target mutations remains a significant hurdle. Although our study did not directly measure off-target effects, the growing body of literature consistently indicates that safety and specificity must be prioritized for CRISPR to be widely accepted in clinical practice.

The delivery mechanisms for CRISPR also remain a critical issue, as our study aligns with the current consensus in the literature. The viral vectors, especially AAVs, have shown promise in delivering CRISPR components to cardiac cells, as reflected in studies like those by [52], which demonstrated successful gene editing in cardiac tissues using AAV-mediated CRISPR. However, the limitations of viral vectors, including payload size restrictions and immune responses, were identified as challenges both in our study and in prior research, indicating that more work is needed to refine delivery strategies to ensure effective and safe gene editing in cardiac cells.

Furthermore, while the technical barriers are significant, the ethical implications of applying CRISPR-Cas9 in human genetic modification, particularly in germline editing, must not be overlooked. Previous research by [53] has highlighted the ethical challenges associated with germline gene editing, including concerns about unintended societal consequences, such as the potential for creating genetic inequalities or the "designer baby" issue. Although this study primarily focused on somatic cell gene editing in cardiac diseases, these ethical concerns remain highly relevant, especially as CRISPR technology progresses from animal models to human clinical trials [54].

In conclusion, the results of this study support the growing body of literature on the potential of CRISPR-Cas9 in revolutionizing the treatment of genetic heart diseases. Healthcare professionals' awareness of CRISPR-Cas9 and the perceived usefulness of the technology were key determinants in shaping attitudes toward its application in clinical settings, a finding consistent with several previous studies [55]. However, the challenges related to safe delivery, off-target effects, and long-term safety remain critical issues that require further research and development. As CRISPR technology continues to evolve, it is imperative that these technical and ethical concerns are addressed, ensuring that the promise of gene-editing therapies is realized safely and effectively. The positive shift in attitudes observed in this study highlights the potential for CRISPR-Cas9 to play a key role in the future of cardiovascular gene therapies, particularly for inherited heart diseases [56].

CONCLUSION

This study explores the potential of CRISPR-Cas9 technology in the genetic modification of cardiac cells, with the goal of offering a novel approach to treating inherited heart diseases. The results demonstrate that the awareness of CRISPR-Cas9 and the perceived usefulness of the technology among healthcare professionals are significant predictors of their attitudes toward its application. Healthcare professionals who were more aware of CRISPR-Cas9 and recognized its potential benefits were more likely to have favorable attitudes toward integrating this technology into clinical practice. This finding emphasizes the importance of increasing knowledge and education around CRISPR technology, especially in fields related to genetics and cardiovascular medicine. It suggests that promoting awareness and understanding of CRISPR-Cas9 could help to overcome potential hesitations and encourage its clinical adoption in the treatment of genetic heart diseases, such as hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM) [57].

Despite the promising outlook, several challenges remain in the practical application of CRISPR-Cas9 in cardiovascular medicine. While viral vectors like adeno-associated viruses (AAVs) have shown success in delivering CRISPR components into cardiac cells, the issues of off-target effects, payload size limitations, and the immune response still pose significant hurdles. These findings are consistent with the broader body of literature, which acknowledges that, although CRISPR-Cas9 holds immense potential, its application in clinical settings requires further refinement to ensure its safety and efficacy. The technical challenges of gene delivery and the need for higher precision in targeting specific mutations remain key obstacles to the widespread use of CRISPR for genetic modifications in cardiac cells. Future research should continue to address these concerns by developing more advanced delivery methods and improving the specificity of CRISPR-Cas9 to minimize unintended genetic alterations [58].

Furthermore, while the clinical potential of CRISPR-Cas9 is highly promising, ethical considerations surrounding the use of this technology in human genetic modification must not be overlooked. The potential for germline editing and its implications on future generations raises significant ethical concerns, particularly regarding the long-term societal effects of such interventions. As this technology moves closer to clinical use, it is essential that ethical guidelines and regulatory frameworks are developed to govern its application, particularly when it comes to germline genetic modifications. It is also important to consider the accessibility of CRISPR-based therapies, ensuring that these advancements are available to a broad population, including those in developing countries like Pakistan, where cardiovascular diseases are a leading cause of death. In conclusion, while the results of this study highlight the potential of CRISPR-Cas9 to revolutionize the treatment of inherited heart diseases, there is a need for continued research, technological advancements, and ethical deliberations before it can become a routine part of clinical practice. As we look ahead, CRISPR-Cas9 could play a pivotal role in reshaping the landscape of cardiovascular medicine, offering hope for patients with genetic heart conditions [59, 60].

Future Implication

The future implications of CRISPR-Cas9 technology in the treatment of cardiovascular diseases are profound, offering the potential to revolutionize the management of genetic heart conditions. As research progresses, CRISPR could enable the development of curative therapies that target the genetic mutations responsible for diseases like hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM), potentially preventing or halting the progression of these conditions before they manifest clinically. The refinement of delivery methods and the reduction of off-target effects will be crucial for ensuring the safe and effective application of CRISPR in human patients, particularly in the non-dividing cardiac cells. Furthermore, as the ethical, technical, and regulatory frameworks evolve, CRISPR could pave the way for personalized medicine, offering treatments tailored to an individual's unique genetic makeup. This innovation could not only enhance treatment outcomes for patients but also offer preventive options for high-risk populations, changing the landscape of cardiovascular care globally, including in regions with high burdens of genetic heart diseases such as Pakistan.

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